mRNAs inside the expressing cells (Zhang et al., supra; Palmiter et al., Nature 300:611-615, 1982; Palmiter et al., Science 222:809-814, 1983; Hammer et al., Nature 315:680-683, 1985; and Selden et al., Mol Cell Biol 6:3137-3179, 1986). The level of GH in wtHS-40 transgenic mice were all low and comparable to non-transgenic controls. This was consistent with observations that the human ζ-globin promoter activity is essentially shut off in adult transgenic mice, even when it is linked in cis with the wtHS-40 enhancer or with the ß-globin locus control region (Pondel et al., Nucl Acids Res 20:5655-5660, 1992; Robertson et al., Proc Natl Acad Sci USA 92:5371-5375, 1995; Albitar et al., Mol Cell Biol 11:3786-3794, 1991; and Spanger et al., Nucl Acids Res 18:7093-7097, 1990).

In contrast, the blood GH levels of the ten founder mice having the mtHS-40 enhancer exhibited a roughly linear, positive relationship relative to transgene copy number. Further, the expression of the mtHS-40 transgene was integration site-independent (i.e., position-independent) because the integration sites here were believed to be random and mice having similar transgene copy numbers exhibit similar level of expression. The blood GH levels these founders at other ages, as well as these founders' progeny, were similar to the levels of expression in mtHS-40-containing mice, as shown in Table 1.

To analyze the GH RNA levels in transgenic fetuses and embryos, liquid N_2-frozen embryos, fetuses, or fetal livers were manually homogenized, and the RNA isolated by standard acid guandinium isothiocyanate-phenol-chloroform extraction (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY, 2nd ed., 1989). For adult samples, the mice were rendered anemic by three injections of phenylhydrazine (40 μg/g of

body weight) so that erythroblasts would enter the adult blood and be collected for analysis. The second injection was 8 hours after the first injection, and the third injection was 24 hours after the first. Six days after the first injection, the mice were sacrificed, and the RNA was isolated from different tissues. In all cases, the total RNA was used for the following assay without further purification.

RT-PCR was carried out as described in Chelly et al., Nature 333:858-860, 1988 and Foley et al., Trends Genet 9:380-385, 1993. Each reverse transcription reaction mixture contained 1 μg of RNA, 200 units of SUPERSCRIPT II™ reverse transcriptase (Gibco BRL), and 20 mM oligo $d(T)_{15}$. One-twentieth of the cDNA was then amplified by PCR using Taq polymerase (Gibco BRL) and primers specific for human GH, mouse β^{major}, mouse ζ-globin promoter, or mouse G3PDH. Amplifications were carried out in a HYBRID OmniGene system with the following temperature profiles: an initial denaturation at 95°C for 3 min, 53°C for 1 min, and 72°C for 1 min; followed by repeating cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and finally an elongation step at 72°C for 5 min. Each PCR analysis was done in duplicate. The sequences of PCR primers used are as follows. For mG3PDH, TGAAGGTCGGTGTGAACGGATTTGGC (SEQ ID NO:4) was used as the 5' primer, and CATGTAGGCCATGAGGTCCACCAC (SEQ ID NO:5) was used at the 3' primer. For the human GH gene, GTCCCTGCTCCTGGCTTT (SEQ ID NO:6) was used as the 5' primer, and ATGCGGAGCAGCTCCAGGTT (SEQ ID NO:7) was used as the 3' primer. Another 3' primer used for the human GH gene was CATCAGCGTTTGGATGCCTT (SEQ ID NO:8). For the mouse β^{major} sequence, TGGGCAGGCTGCTGGTTA (SEQ ID NO:9) was used at the 5' primer, and TTAGTGGTACTTGTGAGCCAA (SEQ ID NO:10) was used as the 3'

primer. For the mouse ζ-globin promoter sequence, CTGATGAAGAATGAGAGAGC (SEQ ID NO:11) was used as the 5' primer, and TAGAGGTACTTCTCATCAGTCAG (SEQ ID NO:12) was used as the 3' primer. The PCR product lengths were 980 bp for mouse G3PDH, 335 bp for mouse $ß^{major}$, and 290 bp or 450 bp for ζ-GH. One-fifth of each PCR reaction was resolved on a 1.5% agarose-ethidium bromide gel, which was then documented using a IS1000 Digital Imaging System and saved as a TIF computer file. The band intensities were quantitated by the PhosphorImage System.

For semi-quantitative purposes, mouse G3PDH was used as the internal standard. The linearity of amplification of the G3PDH cDNA was first defined by amplification of serial dilutions of the cDNA samples. Twenty five cycles were chosen for amplifying mouse G3PDH since, under the reaction conditions described above, the signals were linear over a wide range of dilutions of cDNA. In the initial calibration test, G3PDH bands with similar intensities were obtained from the different tissue cDNA when the same amount of RNA was used for reverse transcription. The appropriate PCR cycle number used to amplify the human GH, mouse $ß^{major}$, and mouse ζ-globin transcripts were 28, 25 and 28, respectively. The amount of different cDNA used for amplification were first determined by PCR using the mouse G3PDG primers, then individual PCR reactions were performed using the human GH, mouse $ß^{major}$, or mouse ζ-globin primers.

It was known that, in the developing mouse, the first site of erythropoiesis is at the yolk sac blood island at 8-14 days of gestation. The major site of erythropoiesis then shifts to the fetal liver, and finally to the spleen at birth. The expression of GH transcripts from the mouse ζ-globin promoter in adult transgenic mice containing the mtHS-40 enhancer was examined. In all adult mice having the